Attachment B

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THE OCEANIC INSTITUTE

An Interim Report

on

RESEARCH IN ARTIFICIAL PROPAGATION OF MILKFISH

and

PROPOSED WORK

to

the Research Advisory Committee of

U.S. AID

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INTRODUCTION

This is an interim report to the Research Advisory Committee of U.S. AID. It is written in the context of a period of 8 months of work on a program whose scope and objectives were modified in May 1977. The results presented describe progress on a program scheduled for completion in January 1980.

The seemingly simple objective of using hormones to induce spawning in milkfish is not so simple when you look at the steps involved. A description of the steps involved in obtaining broodstock and spawning these animals provides a perspective for the accomplishments to date:

- finding the fish
- catching the fish
- handling the fish
- transporting the fish
- holding the fish
- spawning the fish
- fertilizing the eggs
- hatching the eggs
- holding the larvae
- feeding the larvae
- transporting the juveniles (fry)

Developing methods and carrying out each of these activities revealed a whole new set of unsolved problems. The solution of these problems in a practical and repeatable fashion is the minimum requirement for the development of transferable technology that will realistically contribute to the production of fish in any context.

GOALS AND OBJECTIVES

A. Goals

The ultimate goal of this project is to develop methods and systems that will result in the capability to breed milkfish. This will result in the capacity to supply stocking seed for culture ponds in Southeast Asia and other areas where milkfish farming is important to food production. To accomplish this goal, we have identified a list of activities or problems to be solved. These activities are the objectives of the current program; the scope of activities range from finding broodstock to eventually solving the food requirements for larvae. For the purpose of this report, the activities of the program are grouped into seven distinct categories which, when complete, should fulfill the goal of this program. These are the specific objectives:

B. Objectives

1. Establish broodstock in captivity
2. Establish conditions for spawning
3. Induce spawning by hormone injection
4. Establish conditions for larval rearing in the laboratory
5. Develop practical methods for large-scale fry production
6. Determine the economics of hatchery operation
7. Conduct health care and pathology research

I. ESTABLISH BROODSTOCK IN CAPTIVITY

A. Scope

The scope of these activities include:

1. Collect mature-sized fish from wild populations.

2. Collect immature fish and establish populations for spawning in future years.

3. Develop appropriate holding facilities for these stocks.

B. Results to Date

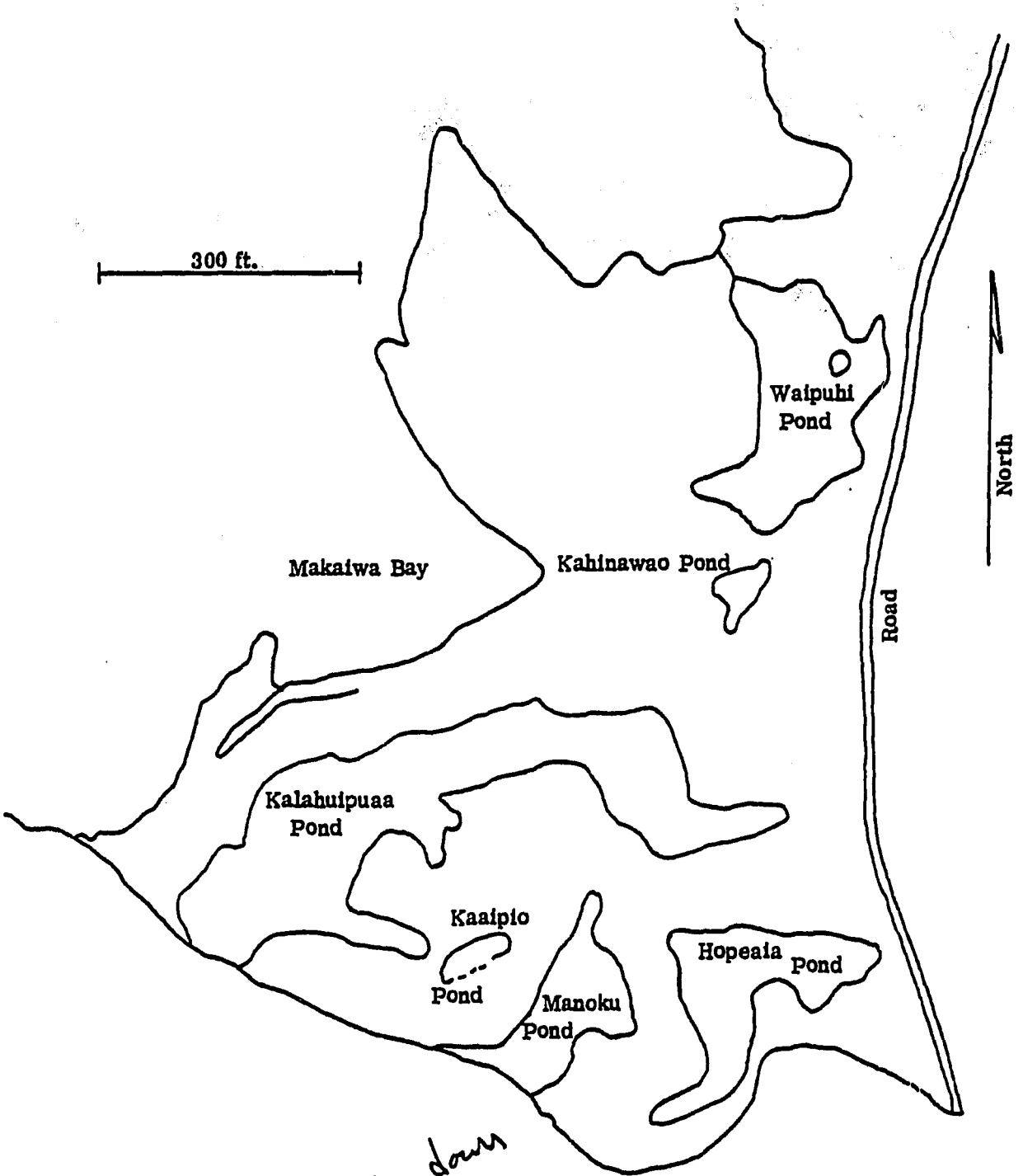
Holding facilities are essential to being able to hold stocks. We have taken the dual approach of developing artificial holding facilities at the Oceanic Institute as well as management of natural pond areas where milkfish are known to reach sexual maturity. These were then stocked with fish. The use of natural ponds involved obtaining permission from owners and managers of the land. Specifically, three ponds are now being used; these are: Hopeaia and Manoku ponds on Hawaii, and Nomilu pond on Kauai. In addition, we have designed and built three experimental environments at the Oceanic Institute. These include four 1/8-acre ponds, one 0.88-acre pond, and a 400 sq. ft. controlled-environment tank.

1. Fishing Program

To provide stocks for these ponds and tank, the Oceanic Institute has, in cooperation with Alu Like (who have provided the manpower for the program), developed a fishing team to capture wild stocks in Pearl Harbor, Kaneohe Bay, and other ocean locations. This team, consisting of five persons, has been successful in locating several wild populations and has developed capture methods for milkfish juveniles and subadults.

a. Juvenile Fish. The methods of capturing juveniles are relatively straightforward and survivals in excess of 70% are routinely achieved. To date, we have captured 805 immature fish with sizes ranging from 1.3 cm to 40 cm.

b. Mature Fish. The size at which a milkfish reaches sexual maturity is dependent upon the environmental conditions; age is also a determining factor for this species. For example, in Hawaii and the Philippines, a mature milkfish is usually about 1 meter long and weighs in excess of 14 pounds, in comparison with Christmas Island where mature fish are found in salinities ranging



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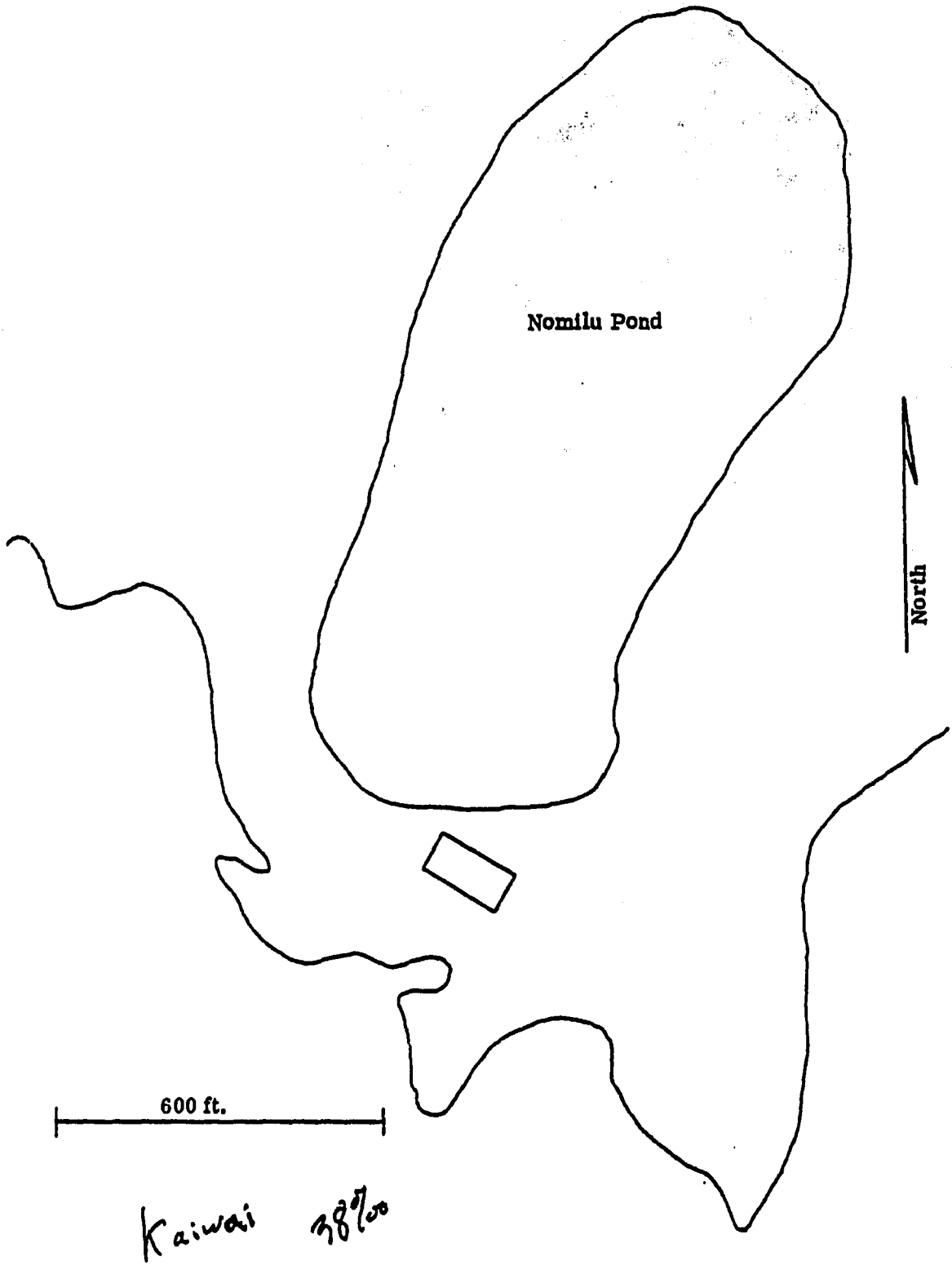

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from 76 ppm to 102 ppm and reach maturity with lenghs of 0.38 m and weighing 2.2 pounds.

Capturing mature fish in ponds at Christmas Island and pond locations in Hawaii has been relatively successful with about 90% survival. By comparison with Christmas Island, the number of mature fish in ponds in Hawaii is relatively small. Transportation from neighbor islands to Oahu is logistically difficult and expensive. Thus we have used fish on Kauai and Hawaii for pond studies and developed artificial environments on Oahu where the Institute is located. The following is a summary of the livestock in captivity.

The subadults stocked last year in the Lahuipuaa pond complex on the island of Hawaii were examined in July. The growth of these fish was found to be satisfactory and they can be used for induced breeding in the 1979 season.

In addition to these 77 stocked subadults, recruitment of subadults to this ›ond complex is continuing. A total of 360 subadults and 115 juveniles have been ıtocked in Hopeaia and Manoku ponds this year.

The fishing effort for milkfish is continuing and intensifying in Pearl Harbor nd Kaneohe Bay during the breeding season of 1978. This is intended to increase ıe size of milkfish stock in captivity. They will be used for growth and maturaon studies in the coming year(s).

A total of 150 subadults and 650 juveniles were captured this year. As of ıgust 1978, milkfish inventory are:

(1) On the Oceanic Institute campus:

a. Rubber-lined ponds: 19 adults, 74 subadults, 650 juveniles

b. Environmental tank: 10 adults

c. New dirt pond #2: 0 adults, 11 subadults

d. New dirt pond #3: 13 adults, 47 subadults

e. Beer vats: 2 adults, 29 subadults

(2) Off campus:

a. National Marine Fisheries Service, Kewalo: 6 adults

b. Kualoa pond: 15 adults

c. Kaneohe Ranch pond: 30 subadults

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(3) Neighbor islands:

a. Lahuipuaa pond complex, Hawaii: 30 adults, 433 subadults, 400 juveniles

b. Nomilu pond, Kauai: 30 adults

(4) Totals:

a. 1977: 32 adults,* 74 subadults*

b. 1978: 125 adults, 624 subadults

Fry were again caught by a fine seine net at Hawaii Kai, Oahu, during several field trips in the months of July and August. All of the fry exhibited the elongation and transparency characteristics of new recruits to the estuarine population. The length of the fry at the time of collection was 1.3 - 1.6 cm (1.5 cm average). They were used for transport experiments and then for the growth studies.

2. Holding Facilities Development Program

a. New Facilities

To accommodate the increasing milkfish stock at the Institute, new holding facilities have been added. They are:

(1) A large indoor tank. It is a rectangular concrete block tank measuring 5.2 m x 4.3 m x 1.2 m deep with a volume of 29.6 m^3. Two collecting channels, 4.3 m x 0.6 m deep, were incorporated into the bottom of the tank to facilitate the catching and handling of the fish. The photoperiod, salinity, and temperature are controlled. Its purpose is to test diet and environmental control for stimulating gonad maturation. This system will hold 20 or more large milkfish. At present there are 10 fish in this system.

(2) Four 1/8-acre by 4 ft. deep outdoor dirt ponds have been completed and brought to productive condition. They have both fresh and saltwater supplies and discharge gates for trapping fish by lowering the pond level. They are general-purpose ponds which were constructed with private funds, and they are available for milkfish growth and maturation trials.

*Adults are fish older than 4 years, and subadults are those 2 to 3 years old.

(3) A new 0.88-acre by 6 ft. deep dirt pond was constructed. The pond construction and catchment gate are similar to the ponds mentioned above. This pond is exclusively for the milkfish program and will be used to study maturation and carry out nutrition studies.

b. Existing Holding Facilities

(1) Six butyl rubber-lined ponds, 20 ft. x 30 ft. x 3 ft. (L x W x D) have been used to stock mullet and subadult milkfish. These ponds will be used for nutrition studies prior to the construction of the new pond.

(2) A dirt pond in the neighborhood of 0.88 acre has been used to maintain broodstock. This pond will be converted to an oxygenated pond when the water system is completed.

These new facilities increase the holding capacity from 7,200 cu. ft. to 250,000 cu. ft. and remove a critical limitation we have suffered in the past.

3. Handling and Transport Program

Upon capture, the procedures for handling and transport have been established. The fish were handled by either dipnet or water-filled plastic bags. To minimize the handling stress and damage, the latter is preferred. An 800-liter circular tank with a firm cover is used for transporting the milkfish. By reducing the salinity and providing strong aeration, the best results are achieved.

Newly captured fish almost always have some injury. We have found that holding fish in a 16,000-liter tank and treating with Furacin greatly decreases the incidence of infection and mortality. A salinity of 18‰ is maintained throughout the treatment period. The fish are treated with Furacin or Furan 2 (+)(-), once a day for 10 days or more until all visible lesions or abrasions are healed. The length of treatment is dependent upon the amount of handling damage and the recovery time required for the particular fish. When the fish has recovered, this procedure is followed by another 4 days' acclimation period during which the salinity is adjusted to the natural condition prior to the distribution of fish into the permanent holding tanks or the experimental environment to which the fish will be transferred.

II. ESTABLISH CONDITIONS FOR SPAWNING

A. Scope

Under this section of the work, we have taken the approach of developing conditions which mimic the environments where milkfish were known to achieve sexual maturity under natural conditions. We have assumed that the environmental conditions in which milkfish with mature eggs are found are suitable for gonadal maturation. Fish with mature ovaries have been found in several locations: Kauai, Hawaii, Oahu, and Christmas Island. On the basis of these observations, we have decided to emphasize a two-pronged approach to spawning milkfish in captivity: establishing stocks in environments which are known to be suitable; and building environments that can be managed to mimic the conditions where the milkfish matured to spawning condition.

B. Results

1. Environmental Regulation of Captive Stock

Intensive fishing efforts are being directed at obtaining additional milkfish adults for induced-spawning trials and to increase broodstock. In captivity, resident milkfish have been subjected to a variety of modified environments in order to evaluate the conditions required for gonadal maturation in captivity.

One of the photoperiod laboratories at the Oceanic Institute has been converted to a controlled holding facility. A total of 10 adult milkfish were stocked and the environmental conditions have been maintained at a constant photoperiod of 18L/6D, a temperature of 25° C, and light intensity averaging 7.5 footcandles. Naviculoid diatoms and enteromorpha have been provided as natural food sources in this concrete tank. In addition, the diet is supplemented with catfish chow and protein supplement in the form of processed *Spirulina*. 3'x6' tank

The gonad development of these fish was examined after 10 weeks (on July 11). No development of eggs had occurred at that time. Since the time was short between stocking and sampling, the study is still in progress and the fish will be sampled again in 10 weeks.

2. Ponds

We are using Hopeaia and Manoku ponds where previous work with hormones resulted in release of fully hydrated eggs as a location for captive stocks. These ponds have 50 resident adults and 77 subadults that should reach maturity next breeding season.

We have made arrangements to use two ponds on Oahu as sources of mature milkfish for future years. We have stocked one of these ponds in the past and feel confident that this environment will continue to produce mature females. Since mature females tend to leave brackishwater environments and head for the sea as the time of spawning approaches, fish which come to the gates between the pond and the sea can be assumed to be mature. Intensifying our efforts to retrieve fish from the gates is expected to be a productive approach in years to come. This behavior may be a bioassay for maturation in captivity.

As described in the previous section, we have built and stocked a number of ponds at the Oceanic Institute. The 1/8-acre ponds can be controlled for salinity and food. 7‰

With the expansion of fish-holding facilities at the Institute and on Oahu, we expect to be able to provide strong logistics support to the spawning program in the next year.

3. Nutrition

We have recognized that nutrition plays an important role in its contribution to the maturation process and the composition of the eggs. However, until we were in a position to have sufficient stocks of immature and maturing and mature fish and manageable facilities, it was premature to start on a nutrition program.

In starting on a nutrition program, we have assumed that in locations where milkfish farming would be supported by a hatchery program the broodstock would probably be held in ponds. Thus we have attempted to extrapolate from pond observations and consider pond management as part of the nutrition program. This year, only a few preliminary steps were taken in the area of nutrition. We anticipate that in 1979 we will be in a position to carry out an experimental program in this area.

Since milkfish were shown to sexually mature in natural ponds by grazing on indigenous foods, we have taken a preliminary approach to nutrition by transplanting species of algae that milkfish are observed to be using as feeds to OI ponds and maintaining outdoor environments which mimic the ponds where sexual maturation has been demonstrated. Providing supplemental feed by transplanting the species of algae and aquatic plants observed to be natural food for maturing animals may be productive. This work is now in progress.

Since we have captured significant numbers of subadult fish, it may be possible to evaluate various feeds and supplements for juvenile and subadult fish if it is the desire of the committee.

Without adequate nutrition, even the most precisely controlled environmental conditions would not likely produce mature eggs. No information is available on the qualitative and quantitative requirements of the milkfish diet. Previous work by Halver and others has established that even when extensive studies of essential amino acids, lipids, carbohydrates, and vitamins have been completed, diets suitable for reproduction will not necessarily result. Since these kinds of studies require many years and thousands of dollars, we have taken the advice of the 1977 RAC committee and formulated a basal diet consisting of:

Wheat middlings 20% Soybean meal 20% Tunafish meal 60%	Premix--97 parts
Propylene glycol Visorbin (Vitamin B complex) Vitamin premix	3 parts

By feeding this diet to captive populations at the Institute, it will be possible to compare the diets as a supplement to natural nutrition. The more practical food used for these herbivorous captive fish are modifications of the pelletized food used for catfish. During shortages of this catfish chow supply, the diet is then substituted by the above-mentioned prepared feed being developed at the Institute.

III. INDUCE SPAWNING BY HORMONE INJECTION

A. Scope

This part of the program focuses on producing sufficient seedstock in captivity by developing a reliable and defined spawning procedure. A consideration of cost of hormones is part of this approach.

The present state of the art indicates that unless adult milkfish are subjected to environmental conditions which result in the stimulation of gonad development, induction of spawning by hormone injections will not be possible. We have shown that adult milkfish of both sexes can reach sexual maturity in confined brackish water and hypersaline ponds and other coastal environments. At the time of spawning, the fish migrate to sea where the natural conditions trigger spawning and provide an environment that favors larval survival. In this part of the program we are attempting the artificial induction of milkfish spawning by injections of exogenous hormones, as a means of bypassing the unknown environmental requirements, for the last step.

B. Results

Understanding of the reproductive cycle and spawning conditions are essential to control the breeding in captivity.

1. The Reproductive Cycle

Individual statistical data have been accumulated to define the reproductive cycle of milkfish in the Hawaiian waters. The gonadosomatic index (GSI) and the monthly variation of oocyte composition are used to postulate the cycle.

In Hawaii, the breeding season of milkfish occurs in the months of June through August, and September is the postspawning period. The data from the milkfish population in Hawaii suggests a synchronous spawning behavior, namely all the mature oocytes are released at one time and the adults spawn once a year. The details of the reproductive cycle are shown in Appendix A.

2. Work on Induced Spawning

47 To exploit an alternative milkfish population prior to the beginning of the breeding season in Hawaii, fieldwork on induced spawning was conducted on Christmas Island from April 20 through June 7, 1978. The report on the fieldwork on Christmas Island is attached (Appendix B).

To conduct fieldwork on induced spawning in the neighbor islands (Hawaii and Kauai), five trips were made in this breeding season. The trips were:

a. June 26 - July 1, 1978: Hopeaia pond, Hawaii

b. July 17 - July 22, 1978: Manoku pond, Hawaii

c. July 25 - July 29, 1978: Nomilu pond, Kauai

d. August 7 - August 16, 1978: Nomilu pond, Kauai

e. August 28 - September 1, 1978: Hopeaia pond, Hawaii

The details of each trip are described as follows:

a. June 26 - July 1, Hopeaia pond, Hawaii

Over 100 milkfish adults were trapped in Hopeaia pond and examined. Most of them are sexually immature and they were presumably stocked in the previous year. Only five maturing or mature females were available; two of them were at the stage of yolk globule, with a mean egg diameter of 0.62 mm, and the other three females were still far from sexual maturation. The mean egg diameters were around 0.32 mm.

All the males from the original broodstock were again sexually mature.

Previous results indicated that the oocytes should be 0.7 mm in diameter or above to offer the greatest chance for successful induced breeding. A priming dose of homogenate is injected on capture. This has been shown to be most successful when it is followed by a minimal number of injections of gonadotropin in the ensuing period. It should be noted that although an individual female fish may be in the maturation phase on capture, the number of low-level daily injections of hormone required to advance development may unnecessarily stress the fish and possibly kill it. Thus when the oocyte diameter is smaller than 0.7 mm it is necessary to wait for female fish to undergo further vitellogenic development before injecting the hormone.

For this reason, only two females were subjected to three injections, 50 mg CPH and 5,000 IU HCG daily for three days, to examine the oocyte response to the hormone treatments. The response was not significant and they, along with two other females, were released back to the Hopeaia pond.

b. July 17 - July 22, Manoku pond, Hawaii

Only one mature female, with eggs of 0.55 - 0.6 mm, was captured. Running males were obtainable from the pond. Because of the maturation state of the female, no attempt was made for induced breeding trials.

c. July 25 - July 29, Nomilu pond, Kauai

This trip was made primarily to evaluate the potentiality of milkfish populations in Nomilu pond for induced breeding use. The pond, about 26 acres with a population of about 30 milkfish adults, presently is not in use. The purpose of this trip was to examine (1) the feasibility of capturing milkfish adults, (2) gonadal development of milkfish adults, and (3) the possibility of establishing a fieldwork site.

Because of the size and depth of the pond, a method of trapping is used. A seine net, 300 x 18 ft. (L x D) with a pulley system was used to intersect the 1/6 section of the pond; fish are then trapped in the pocket. A total of five adults were trapped: four mature females and one running male. Only two females with eggs larger than 0.8 mm were injected with 100 mg CPH and 5,000 IU HCG as a primary dose. This was followed by 100 mg CPH and 20,000 IU HCG each on a daily basis. Since the Kauai work was a new location for the team, almost no support facilities were available at this location.

A temporary holding facility was set up by enclosing a small section of the pond. It was inadequate to maintain the milkfish adults, as it was too shallow and muddy.

After two injections, one of the females released eggs. The second female died of handling stress and infection. Since these first results seemed to offer promise, we decided to establish a working facility on Kauai to work on this captive population. A second trip to the Nomilu pond was planned.

d. August 7 - August 16, Nomilu pond, Kauai

A temporary holding tank with necessary equipment was set up on site. The fishing operation, as in previous trips, was conducted to capture milkfish adults.

The milkfish were captured two times from a total of five fishing trials. In the first catch, only four mature females were caught; the egg diameters were 0.6 and 0.8 mm (two fish each). No males were captured at this time. Two of the captured females, with eggs of 0.8 mm in diameter, were injected with 25 mg CPH and 2,500 IU HCG. One of the treated females released the eggs in the holding tank 22 hours after injection. Unfortunately, since no males were captured,

fertilization was impossible. The remaining two females proved to be too immature to be injected (egg diameter of 0.6 mm). No injections were given to these females and they were then released.

Further efforts were made to obtain ripe males to continue the induced-breeding trials. The fish soon learned to avoid the net and in this deep pond, our equipment was not adequate for further fishing. All the captured females were then released back to the pond after four days in captivity.

A second attempt to catch milkfish adults was initiated. Only one ripe male was caught. Continued fishing failed to capture any female for induced-breeding trials; therefore, the fieldwork at Nomilu pond, Kauai, was terminated.

Several comments may provide important perspective. The fish that spawned produced a full complement of fully hydrated eggs. There is every reason to believe that if a mature male or cryogenically preserved sperm were available, fertilization might have been accomplished.

This almost result emphasizes the need for more organization and preplanning. We feel that the captive populations now established will largely overcome these problems. Cryopreservation of sperm may be useful for backup support to the programs.

e. August 28 - September 1, Hopeaia pond, Hawaii

The gonad development of the resident broodstock in Hopeaia pond was reexamined. Most of the males were still at the running condition. Although the number of original broodstock identified in 1975 was numerous, losses occurred mainly through poaching, with smaller losses from handling and an early shipment of 30 adults to OI offset by recruitment from subadults and juveniles in the subsequent years.

Two females, not fully mature (0.32 mm in egg diameter) at the end of June, were recovered on this trip. One of them was full of atretic eggs, while the other showed significant progress in the ovarian development (vitellogenesis). The egg diameter increased from 0.32 mm to 0.62 mm in two months. These eggs were full of yolk materials, yet they were smaller than the optimal egg size proposed for induced spawning trial; 0.7 mm or above was reported to be an adequate size range.

The experimental adults were acclimated in 7‰ brackish water for a few hours after their capture from the pond where the salinity is 7‰, and the salinity was gradually adjusted to 35‰ in 30 hours. A total of seven injections of carp pituitary homogenate (ranging from 100 mg to 200 mg each) and HCG (ranging from 5,000 IU to 20,000 IU each) were given. The schedule of two injections daily, 9 a.m. and 6 p.m., was followed. 7

At the end of four days' injection period, the fish conditions were excellent. However, no response of the yolk-laden eggs to the hormone treatments was accomplished. From these observations we can conclude that progress of ovarian eggs from the early phase of yolk globule stage to the late phase of yolk globule stage is possible, even when sampling stress is introduced. This requires approximately two months. However, yolk-laden eggs smaller than 0.7 mm in diameter are inadequate for hormone-induced spawning.

f. At OI

A total of 15 milkfish adults were obtained from Kaneohe Ranch pond owned by Mr. Ueymura. Two females were found to be mature (egg diameters of 0.83 - 0.87) and ready for induced-breeding trials. Another four adults were identified as males, although they were not fully ripe.

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Both females were injected with carp pituitary homogenate combined with human chorionic gonadotropin twice a day. The times of injections were 3 hours after sunrise and the interface between light and darkness. At these times, the female is more receptive to the hormone treatments (obtained from the work on the mullet, refer to Appendix C).

Injections in one of the females were terminated after four treatments because of the physical damage and appearance of atretic eggs. Induced-breeding trials on the remaining female were continued for two more days. The progress of oocyte hydration was satisfactory and at least 16% of total oocytes were completely clear and ready for fertilization as determined by microscopic examination 12 hours prior to stripping. The stripping was made when the fish showed continuous dribbling. Fertilization of these eggs was impossible because no semen was obtainable from the males (possibly more justification for a sperm cryopreservation activity).

In summary, limited numbers of milkfish adults were available for breeding trials. The mature adults were often collected from either brackish water (7 ~ 12‰) or hypersaline water (76 or 102‰ at Christmas Island).

Acclimation of these fish to the natural breeding condition, presumably 35‰, imposes osmotic stress to the breeders being handled. The salinity adjustment is usually completed in 24-30 hours. The milkfish available in the Philippines may overcome these problems. We feel that the successful demonstration of hormone-induced spawning of fully hydrated eggs, even without fertilization, provides a good foundation for next year's work.

3. Future Work

The major failing of this year's activities was the inability to capture fully developed females and males at the same time. This is especially true in the fieldwork in the neighbor islands.

Establishing broodstock in captivity is the key to successful artificial propagation of milkfish. Although wild fish can be captured and fertilization of eggs completed following killing and stripping, the long-term benefits will be best obtained through management of the broodstock in captive situations.

We feel that now the establishment of realistic-sized facilities and captive populations of presexed fish should enhance the probability of succeeding in achieving fertilization and carrying out the further studies.

Stocking of juvenile fish in ponds is priority.

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IV. ESTABLISH CONDITIONS FOR LARVAL REARING IN THE LABORATORY

A. Scope

Larval culture involves fertilization, hatching, and then rearing larvae to the juvenile stage. In general, it is essential to maintain optimal environmental conditions and to provide foods suitable for larval growth and survival. The following subobjectives have been defined:

1. To develop larval-rearing techniques with descriptions of physical and food requirements of milkfish larvae, and

2. To improve larval survival by finding the optimal conditions of larval life.

B. Results

Although we have not achieved a fertilization this year, four successful spawnings which produced a full complement of hydrated eggs suggest that we are one step away from fertilization.

1. Food and Physical Systems

Considerable experience in rearing larvae of the grey mullet (*Mugil cephalus*) has resulted in development of facilities and food-production capacity for all stages envisioned as required for larval culture. Foods envisioned to be necessary are phytoplankton and rotifers in culture and *Artemia* nauplii. A review of our facilities should be given at this point.

2. Present Hatchery

The present experimental hatchery at the Oceanic Institute consists of tanks for the growth of algae, phytoplankton, rotifers, and brine shrimp. The hatching facilities allow holding of 10 mullet with four indoor culture tanks with a capacity to rear 15,000 mullet larvae to stocking size. The other six outdoor culture tanks are also available.

3. New Facilities

The above hatchery was adequate to carry out research, development, and demonstration of methods of spawning and larval culture for mullet. However, this facility is clearly inadequate for production of levels of fingerlings that would allow economic evaluation. Recognizing this failing and realizing the necessity of

having a production hatchery as well as facilities that support holding broodstock for large milkfish, considerable expansion of facilities was required. This has been accomplished during the past year. The new hatchery will make the required economic evaluation possible.

4. Hatchery

A production-size hatchery facility was designed which would make use of an existing unfinished building. In August 1978, the governor of Hawaii signed the order releasing the funds for finishing the building; $300,000 of State money coupled with $150,000 of funds from the Doherty Foundation are committed to the program. This provides matching funds of $450,000 to this program.

5. Larval Rearing

Efforts have been made to develop techniques for larval rearing by using wild milkfish larvae before the larvae production is realized in captivity. Collection of newly recruited fry from the wild continued to conduct experiments on larval survival and growth. Various feeds including Spirulina supplement were examined.

The larvae were caught near Hawaii Kai in brackish water and were estimated to range in age from 10-14 days. Total length at this stage averaged 15 mm (range 13-16 mm) and average weight was 13 mg. Fifty larvae were placed in each of six 80 l aquaria with a water exchange rate of 3/day. Other parameters measured in this experiment were salinigy (32‰), temperature (25-26° C) and dissolved oxygen levels (7.0-7.5 mg/l). Aquaria were cleaned daily and rations, mortalities, and other observations were noted. The larvae in each tank were fed one of six different rations daily so that food would not be a limiting factor.

Treatment and results are described as follows:

Treatment	Daily Ration	Total Length (av.) (range)	Weight (av.) (range)	Survival Rate (%)
Chlorella salina	10^4 cells/ml	-- --	-- --	0
Brachionus plicatilus	5 rot./ml	27 mm (18-39 mm)	0.25 g (0.12-0.37 g)	10
C. salina + B. plicatilus	10^4 cells/ml 5 rot./ml	23 mm (18-32 mm)	0.13 g (0.04-0.28 g)	54

Treatment	Daily Ration	Total Length (av.) (range)	Weight (av.) (range)	Survival Rate (%)
Purina catfish chow	0.5-1.0 g	31 mm (25-37 mm)	0.20 g (0.10-0.29 g)	12
Spirulina maxima (spray-dried)	0.5-1.0 g	34 mm (28-45 mm)	0.33 g (0.15-0.73 g)	34
S. maxima + chow	0.5-1.0 g	30 mm (17-40 mm)	0.26 g 0.04-0.58 g)	66

There was a rapid decline in the numbers of larvae fed only the alga Chlorella salina and that treatment was terminated on day 12 when there were no remaining survivors. The other treatments were continued for the full 30-day period. By day 30 the survivors from all of the treatments were noticeably smaller than those caught at the same time but which were stocked in an outdoor pond. These fish were fed supplementally with a 1:1 mixture of spray-dried Spirulina maxima and finely-ground Purina catfish chow.

These preliminary studies demonstrated that Spirulina maxima and S. maxima mixed evenly with finely-ground Purina catfish chow can be successfully used to feed milkfish larvae of 10 or more days. These diets provide good growth and survival and are easily handled and stored at room temperature. It would appear, however, that these rations might be used most effectively as a supplemental feed to natural biogrowth in outdoor ponds rather than as a sole source of nutrition. Further feeding trials are necessary to ascertain whether S. maxima and Purina catfish chow are nutritionally complete for milkfish larvae at this stage.

In preparation for larval fish-rearing efforts with milkfish, the food production system was scaled up to meet future demands. Emphasis has been placed upon improving and expanding phytoplankton and rotifer culture techniques. The ubiquitous algae, Chlorella spp. and Tetraselmis spp., have been selected for their high productivity and ease of culture in large volumes. Brachionus plicatilus was cultured using Chlorella achieving densities of 150 rotifers/ml in 10 days. Tetraselmis spp. was demonstrated to be acceptable to three-week-old milkfish larvae.

A new alga species, Nannochloris spp., is now being mass-cultured for use in milkfish larval rearing. This alga appears to be a superior base for our food chain for two reasons: it can be intensively cultured under a wider temperature range than the Chlorella and Dunaliella previously used, and it has a smaller percentage of cell wall than does the Chlorella, making it easier to digest.

6. Fry Transport

The transport experiments of milkfish fry up to the density of 100 fry per liter were conducted in 1977. The results are shown in Appendix D. The The experiments continued this year when new recruits of milkfish fry became available from the wild. The densities from 50 to 1,000 fry per liter were examined. The results indicated that up to 1,000 10- to 14-day-old fry per liter can be safely transported for periods as long as four days. Salinity was 18‰ in a ratio of 5:1 (air to water).

Results of fry transport experiments for a 7-day period are as follows:

Density	Initial D.O. (ppm)	Mortality (%)	Final D.O. (ppm)	Temperature (° C)
50 fry/l	7.5	2	5.1	24
100 fry/l	7.5	3	4.9	24
500 fry/l	7.5	1.2	2.5	24
1,000 fry/l	7.5	1.1	4*	24

*In the preliminary test, 100% mortality was noted at the end of the 7-day experiment period. The test was repeated and terminated with only 1.1% mortality observed at the end of four days. The loss of the first trial was due to bag leakage.

V. DEVELOP PRACTICAL METHODS FOR LARGE-SCALE FRY PRODUCTION

A. Scope

To develop practical rearing procedures for large-scale production of milkfish seeds through understanding of ecological and nutritional requirements of postlarvae.

The scope of this work starts with the recognition that methods that are developed at a well-equipped research institution are dependent on pumps, filters, and expensive equipment. These methods must be adapted to a set of simple procedures that can be scaled to production-level activities.

B. Results

The completion of this objective will follow from an understanding of the requirements for culture of the milkfish larvae. We have not succeeded in obtaining fertilized eggs this year.

We have made arrangements to interact with the workers at SEAFDEC where the spawning and fertilization of milkfish has been accomplished. Although large numbers of fish have not been produced, we anticipate that based on our past experience with mullet culture, it may be possible to plan for large-scale culture.

However, until specific larval foods, stocking density, flow rates, etc., can be determined, it will not be possible to go any further with this study area.

VI. DETERMINE THE ECONOMICS OF HATCHERY OPERATION

A. Scope

Operating a larval-rearing program of significant size is essential to determine the economics of such an activity. In the absence of a fertilization this year, we cannot carry out this objective at this time.

B. Approach

We will work with the SEAFDEC team to review their program and develop a preliminary cost analysis for the economics. We are working with James M. Montgomery Consulting Engineers who have considerable experience in construction in Southeast Asia to develop preliminary estimates of costs of operation from existing data.

VII. REPORT ON MILKFISH PATHOLOGY PROGRAM

Basic to the spawning and rearing efforts of milkfish in captivity is maintaining them in a healthy condition. This requires in the aquaculture program a pathology/health component. Because of the youthfulness of pathology in the aquaculture field, a program in this area should be broad and diverse. This is the case with the program here at the Oceanic Institute, which has made the following contributions:

I. To milkfish health directly

A. Provided first description of a pathologic condition, gastritis, in the milkfish. The gastritis may be an etiologic factor in producing the well-known nervousness of the milkfish, or the nervousness may be the cause of the gastritis. Paper in press: Smith, A.C. Pathology and Biochemical Genetic Variation in the Milkfish, Chanos chanos. J. Fish Biol.

B. Developed a trio of simple noninvasive tests for identifying early nonspecific stress, stress related to starvation, and stress related to the early onset of disease. Papers published: (1) Smith, A.C., and Ramos, F., 1976. Occult Haemoglobin in Fish Skin Mucus as an Indicator of Early Stress. J. Fish Biol., 9:537-541. (2) Ramos, F., and Smith, A.C., 1977. Ketone Bodies in Fish Skin Mucus as an Indicator of Starvation: A Preliminary Report. J. Fish Biol., 12:105-108. (3) Ramos, F., and Smith, A.C., 1978. The C-Reactive Protein (CRP) Test for the Detection of Early Disease in Fishes. Aquaculture, 14:261-266.

C. Provided first description of allergy-like incompatibilities between milkfish and products (skin mucus) of other fishes. Article published. Smith, A.C., 1977. Reactions of Fish Red Blood Cells with Mucus and Sera from Other Fish(es). Calif. Fish Game, 63:52-57.

D. Determined in milkfish by automated chemistry analysis the baseline serum and skin mucus values for 24 chemical tests before and after the application of stress sufficient to compromise health.* The purpose was to identify (1) the key tests providing health information, particularly with mucus because of its ready availability, and then (2) the simple inexpensive commercially available kit tests for individually carrying them out.

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*Supported by the Rockefeller Foundation and the Engelhard Foundation.

E. Developed from autopsy examinations of milkfish and other fish species a concept of in vivo clotting from multiple etiologies (infection, trauma, etc.) possibly playing an unsuspected major role in causing mortalities.

F. Contributed practical information to field personnel on handling shock-like conditions and other health problems in milkfish related to capture and subsequent containment.

II. To general milkfish biology

A. Developed a simple inexpensive method for identifying intraspecific molecular variation in milkfish. Application of this technique may help to understand the basis for variations in breeding cycles among milkfish of different geographic areas by linking the variations to different subpopulations. Submitted m.s.: Smith, A.C. Reactions of Fish Nuclear Lens Proteins with Sea Cucumber Coelomic Fluid.

B. Developed working models of a simple slide test for identifying the sex of milkfish by blood and skin mucus reactions.

III. To other scientific fields

A. Invertebrate pathology

Demonstrated with the aid of milkfish serum the first known allergy-like reactions in a marine invertebrate. Three papers published: (1) Smith, A.C., 1977. Immunologic Reactions of the Sea Cucumber, Holothuria cinerascens, to Serum from the Milkfish, Chanos chanos. J. Invert. Path., 29:326-331. (2) Smith, A.C., 1978. A Proposed Phylogenetic Relationship between Sea Cucumber Polian Vesicles and the Vertebrate Lymphoreticular System. J. Invert. Path., 31:353-357. (3) Smith, A.C., 1978. Hypersensitivity and Desensitization in the Sea Cucumber, Holothuria cinerascens. Dev. Comp. Immunol., 2: 355-360.

B. Human medicine

1. Developed with the aid of milkfish serum a model of human immunopathologic conditions, viz., bronchial asthma and autoimmune disease. This work is in an advanced stage of writeup.

2. Determined that the milkfish could provide a model of human a- or hypocatalasemia. Published article: Smith, A.C., 1976. Catalase in Fish Red Blood Cells. Comp. Biochem. Physiol., 54B:331-332.

C. Comparative medicine

Described the presence of oncofetal protein (carcinoembryonic antigen, or CEA) in the milkfish. This type of protein is well known in mammals in association with certain types of pathology, particularly malignancy, but had not previously been described in fish. Paper in press: Smith, A.C. Oncofetal Proteins in Marine Animals. Comp. Biochem. Physiol.

VIII. FACILITIES AND STAFF SUMMARY

During the last year, the Oceanic Institute has made a major commitment to marine finfish aquaculture. This is best evidenced in a summary of the facilities directly involved and the milkfish program and the general support laboratories that are involved in the program.

A. Finfish Hatchery

The marine finfish hatchery has been discussed previously. This 13,400 sq. ft. building will have 4,500 sq. ft. committed to larval culture with complete capability for phytoplankton, algae, rotifer, and *Artemia* culture. The building will also have complete capability for water quality analysis.

B. Ponds

The Institute has a variety of experimental ponds available to the program. These range from 20' x 30' rubber-lined ponds for holding juvenile stocks and carrying out feeding experiments to 1/8-acre ponds for conducting experiments with adult and subadult milkfish. The ponds have a full range of water quality and salinity control. Three ponds are equipped with auxiliary aeration systems (these ponds and support systems were constructed with private funds).

C. Photoperiod Control Laboratory

The photoperiod control laboratory contains a 16' x 14' concrete tank. The lab is equipped to control temperature, salinity, and all aspects of water quality and photoperiod. Smaller tanks are also available in similar environments if the program requires these facilities.

D. Support Programs

Water Quality. A fully equipped water quality lab is kept operational at the Institute. Under the direction of Dr. Paul Bienfang and Ms. Wendy Johnson, this laboratory monitors water quality in all programs including microbiological determinations.

Pathology. The pathology program under the direction of Dr. Al Smith provides facilities to autopsy dead animals and diagnose disease situations.

Feed Lab. The Oceanic Institute maintains a basic capability to blend ingredients for the formulation of experimental diets. As the kinds of feeds required are better known, we have been promised support from the Waldron Company which operates a large feed mill on Oahu.

Engineering Services. The Institute has an ongoing engineering program headed by Guy Rothwell. He and Steve Ribakoff are responsible for the design of facilities, tanks, pond water systems. In conjunction with Robert Shleser, they carry out work on economics.

Data Processing. Rosie Rothwell-Koningsberger is responsible for data processing.

Administrative Staff. Richard Power is in charge of the administrative program. Bookkeeping, secretarial services, and drafting are provided to the program.

Library. The library maintains active subscriptions to the major journals that report results in aquaculture, physiology, fish nutrition, and endocrinology.

University of Hawaii. A close working relationship has been developed with the University of Hawaii. William Furtick, Dean of Tropical Agriculture, is one of the trustees of the Institute. John Craven, Marine Affairs Coordinator of the State of Hawaii and Dean of Marine Programs, is also a trustee.